A MOLECULAR DYNAMICS SIMULATION OF NOREPINEPHRINE METABOLITES ON AMYLOID-BETA PROTEINS

A Thesis

Presented to

The Academic Faculty

by

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In Partial Fulfillment

of the Requirements for the Degree

Bachelor of Science with Research Option in the

School of The Wallace H. Coulter Department of Biomedical Engineering

Georgia Institute of Technology

May 2016

A Molecular Dynamics Simulation of Norepinephrine Metabolites on Amyloid-Beta Proteins

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CHAPTER 1

INTRODUCTION

Alzheimer's disease is a type of dementia that causes memory, thinking, and behavior problems usually in people that are 65 or older [1]. It is the sixth leading cause of death in the United States and has affected over 26 million people worldwide [1][2]. In 2050, this number is predicted to increase to 106 million [2]. The cause of Alzheimer's disease are the deposits of Amyloid-Beta (Aβ) protein fragments and the twisted fibers of tau proteins. These proteins build up inside the brain inducing the destruction and death of nerve cells which eventually results in symptoms such as memory failure, personality changes and more [1]. To find a treatment for this disease, many studies have relied on using potential modulators to prevent the formation of the plaques and find potential cures.

Current studies have been able to discover many chemicals that can act as an inhibitory agent to prevent the formation of plaques in the brain cells. Kim and his group measured $Alpha_2$ receptor sites to observe the integrity of noradrenergic system in patients with Alzheimer's disease and found that in subjects with Alzheimer's disease, there was a significant reduction in norepinephrine (NE) concentration [3]. In another study, Heneka and his group performed an *in-vivo* study using mice and discovered that there was an increase in Aβ in NE—depleted mice [4]. However, much of the studies performed *in-vivo* and *in-vitro* are focused on observing the inhibitory effects that these chemicals have on the Aβ protein and not much explanation is given on the exact mechanisms with which these modulators are able to achieve such results. Therefore, a lot of research in the molecular dynamics simulation field is being done to better explain the interaction between potential inhibitors and Aβ proteins.

To our knowledge, no molecular dynamics simulation has been done on norepinephrine to investigate the interaction with Aβ proteins. Hence, the purpose of this study is to observe

and determine the exact mechanism involved in the interaction between 3,4-dihydroxymandelic acid (DHMA), normetanephrine (NMN), which are metabolites of norepinephrine, and amyloid-beta protein through molecular dynamics simulations.

This research has implications for determining potential drugs that are able to interact with Aβ and reduce the protein's aggregating characteristics. Gathering more data on other types of modulatory drugs will better explain how Aβ protein accumulation can be reduced. Furthermore, this research can contribute to current research on Alzheimer's disease and in the long term help find a solution that can potentially cure this dementia.

CHAPTER 2

LITERATURE REVIEW

Increasing research evidence suggests that amyloid-beta (Aβ) protein misfolding and aggregation lead to Alzheimer's disease (AD), a neurodegenerative disease. Accordingly, much of the current research involves discovering potential substances that could prevent aggregation and in turn observe the mechanisms involved during the process. As more research is performed on Aβ, it would be possible to hinder the progression of AD and to even completely cure it in the near future.

Although the exact mechanism is not fully understood, it is known that Aβ proteins, which are helical in their native states, gradually conform into beta-sheet structures and set the ground for the proteins to aggregate and form fibrils that are toxic. These fibrils are associated with Alzheimer's disease. Nerelius et al.[5] investigated the relationship between the α-helical forms of Aβ and neurotoxicity by using ligands to stabilize the helix. Inhibitor ligands, Dec-DETA and Pep1a, were designed to specifically bind to the α-helix of the peptide by using two

methods: *in-vitro* and *in-vivo*. In vitro method involves incubating PC12 cells with Aβ proteins and the ligands, while in-vivo method is fulfilled by feeding transgenic flies with ligand containing fly food. From the experiment, the research group was able to conclude that when Dec-DETA and Pep1a were administered, the α-helical structure of the Aβ protein was retained and consequently, toxicity was reduced in both *in-vitro* and *in-vivo*. On the other hand, Simmon et al. [6] investigated the effects that beta-sheet structures have on the cytotoxicity of neuronal cells. The group incubated Aβ for several days in aqueous solution to let the protein gradually unravel from a helical structure to a β-sheet structure. Then, using the knowledge that the aggregation of the protein is dependent on the β-sheet formation, they measured the toxicity and found that there was a positive correlation between β-sheet structure content and toxicity in hippocampal cells. Due to the combined efforts of several researchers, the effects of the conformational changes of the Aβ protein have been relatively understood. However, the effects and mechanisms that external factors, such as antibodies and chemicals, have on the protein have not been fully explained. Therefore, much of the current research focuses on investigating potential modulators that can prevent Aβ protein aggregation.

Amongst numerous approaches that scientists are taking to find the effects of introducing an external factor to amyloid beta proteins, is antibody administration. The antibodies are able to enter the central nervous system, decorate the plaques formed by the fibrils and induce clearance of preexisting amyloid. Bard et al. [7] intravenously administered 10D5 and 3D6 antibodies to determine whether the antibodies against Aβ had entered the central nervous system and acted directly on the plaques. Through the *ex-vivo* assay of cryostat section of PDAPP mouse and human AD brains, the group was able to conclude that the antibodies against Aβ peptide greatly reduced the plaque deposition and that the entry to the central nervous system was not due to abnormal leakage of the blood-brain barrier. Administration of the antibodies itself is not the only way of targeting Aβ fibrils. Research has

shown that human immunoglobulin contains antibodies against Aβ, which are able to recognize Aβ peptides and reduce Aβ toxicity, as well as inhibit Aβ fibrillation. Consequently, Dodel et al.[8] carried out an experiment on patients with AD by treating them with human immunoglobulin (IVIG) for six months. Through the results, the group found out that IVIG was able to reduce the Aβ concentration in the CSF and conclude that immunoglobulin has the potential to cure Alzheimer's disease. Antibodies have higher specificity to the target, hence a higher efficacy and less side effects can be expected when treating AD [9]. However, the invasive intravenous administration and the high costs encourage researchers to find alternative substances for cure.

The use of chemical substances, as opposed to antibodies to target Aβ proteins, is increasing due to the ease of handling the substances and the use of simpler materials and experiment methods. Wong et al.[10] and his research team used Erythrosine B (ERB) as an Aβ modulator to observe the inhibitory capabilities of the molecule. ERB is a FDA-approved xanthene type of food dye that exhibits no toxicity even at large doses. The Aβ sample was prepared and diluted in the absence or presence of ERB and ThT fluorescence assay, dot-blotting as well as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay were performed to obtain the results. Although the research team was able to conclude that ERB inhibits the formation of Aβ fibrils and therefore prevents aggregation, the exact mechanism involved during this process is currently speculated and not fully understood. Hence, molecular dynamics simulations are performed to better comprehend how such molecules interact with the Aβ protein and ultimately understand its process. As reported by Yang et al.[11], a molecular dynamics simulation was conducted using Leu-Pro-Phe-Phe-Asp (LPFFD) with Aβ peptides. LPFFD is one of the many beta sheet breakers that are designed to prevent the formation of β-sheet structures and amyloid aggregation. The group obtained the protein structures from the Protein Data Bank (11YT) and carried out automated molecular

docking using Autodock 4. Finally, the simulation was performed using GROMACS 3.3.3. MD package with the GROMOS96 43A1 force field. From the results obtained from the simulation, the group found out that LPFFD binds to the C-terminus of the Aβ proteins to prevent the α-helical structure from conforming into a β-sheet structure. Additionally, LPFFD forms hydrogen bonds with the protein structure to stabilize the Aβ and therefore prevent aggregation. Running computer simulations is perhaps the best way to fully understand the mechanism involved in the interactions of various substances with Aβ protein. That is why the use of computer simulations to discover the solutions to well-known diseases, such as Alzheimer's disease is growing.

Most of the research on the prevention of amyloid-beta peptide aggregation involves testing different kinds of substances of different concentrations to see if an inhibitory effect can be observed. However, at the same time, most of these experiments do not explain the aggregation process that is involved. Therefore, the current study focuses on identifying these mechanisms, especially the interaction of 3,4-Dihydroxymandelic Acid (DHMA) and normetanephrine (NMN) with amyloid-beta, through computational simulations based on *in-vitro* experimental results obtained from New York University. The simulation uses Cerius^2, Jaguar, Autodock, and Gromacs to obtain the results that will explain how DHMA and NMN act as modulators. This newly acquired information will help understand Aβ aggregation better and act as a stepping stone to determine other substances that might have an inhibitory effect on aggregation which in turn could be used as potential therapeutic drugs.

CHAPTER 3

MATERIALS AND METHODS

The amyloid beta proteins were taken from the PDB Bank online (Pdb ID: 1BA4). The norepinephrine metabolites, DHMA and NMN, were created using the graphical molecular modelling program Cerius2. DHMA and NMN were then run through Jaguar for density functional analysis (DFT) and charge optimization using the following conditions: Functional theory: B3LYP, Basis: 6-31G, Charge Analysis: Mulliken, Total Charge: -2, and Spin Multiplicity: 1. Next, to calculate the rough binding sites of DHMA and NMN on the amyloid beta protein AutoDock was used. Finally, the MD simulation and analysis were performed using the AMBER 99SB and DRIEDING Forcefields in GROMACS.

CHAPTER 4

RESULTS

4.1 Aβ-DHMA Complex

In order to perform a visual comparison between the structure of the protein without and with the drug, snapshots of the protein with and without the compound were taken at 4 different time pointes shown in Figure 1 and Figure 2. Without the drug, at 0 nanoseconds the Aβ protein retained its normal alpha helical structure, at 50 and 100 nanoseconds the protein gradually started to straighten and uncoil and finally at 150 nanoseconds the Aβ protein completely lost its helical structure. With the addition of the drug, at 0 nanoseconds the Aβ protein is at its helical structure, at 50 and 100 nanoseconds the Aβ protein gradually loses its helical structure but it still maintains it and at 150 nanoseconds the drug completely detaches itself from the protein.

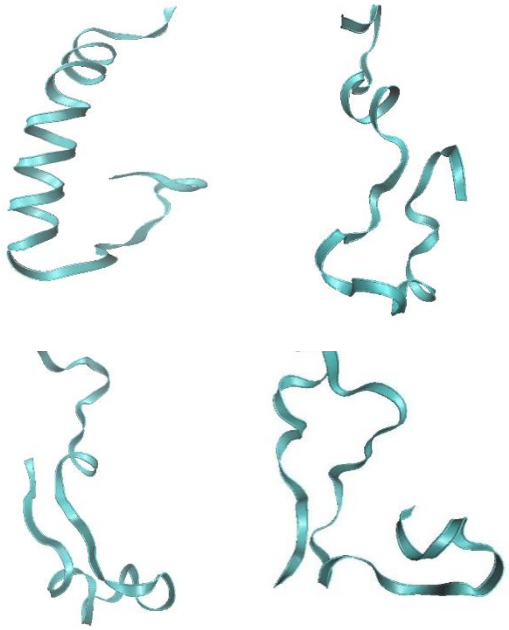

Figure 1 Aβ protein structure. The change in morphology of the protein at four different time points, 0ns (top left), 50ns (top right), 100ns (bottom left), 150ns (bottom right), is shown.

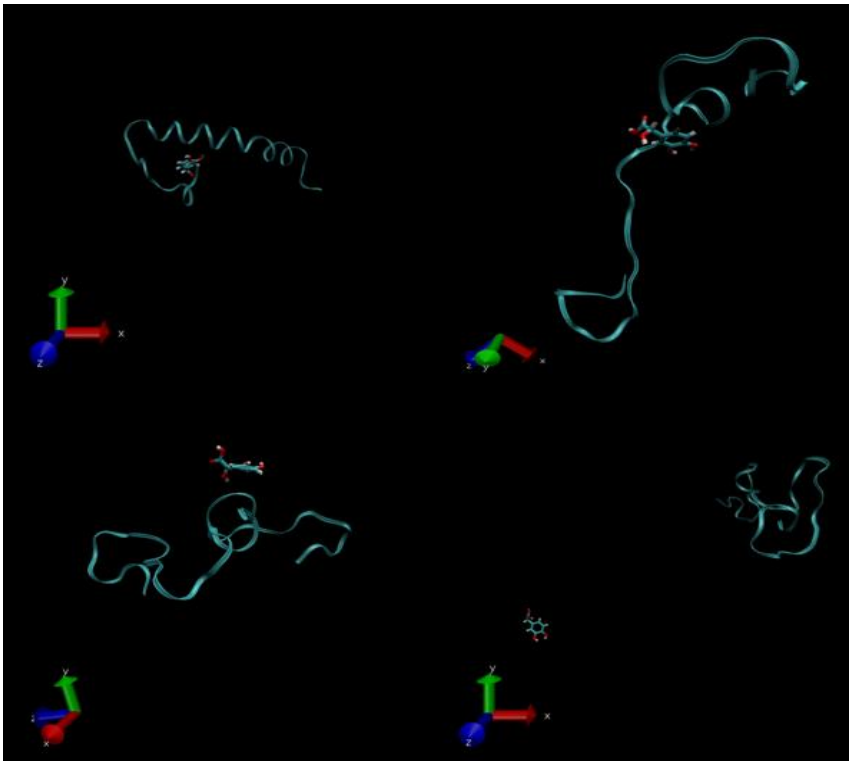

Figure 2 Aβ-DHMA complex. The change in morphology of the protein with DHMA at four different time points, 0ns (top left), 50ns (top right),

4.2 Conformational Change of Backbone During MD Simulation

The Root Mean Square Deviation (RMSD) analysis was performed to observe the change in the structure of backbone of the Aβ protein with and without DHMA through time as shown in Figure 3 and Figure 4. The RMSD of the Aβ protein with decreased compared to the RMSD value of the Aβ protein alone, indicating that DHMA was effective in minimizing the deformation of the protein.

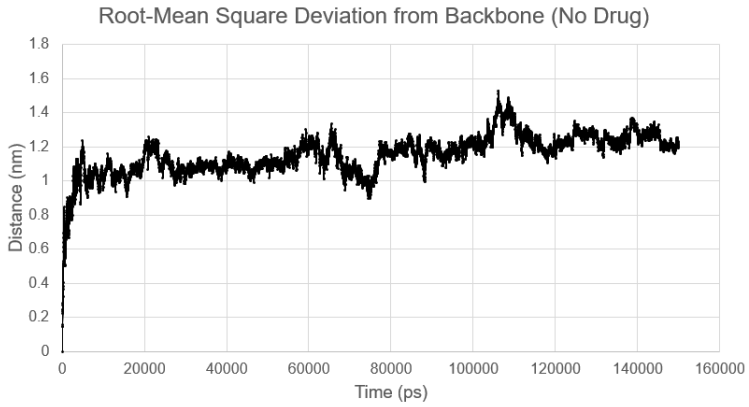

Figure 3 Time averaged distance between Aβ without DHMA.

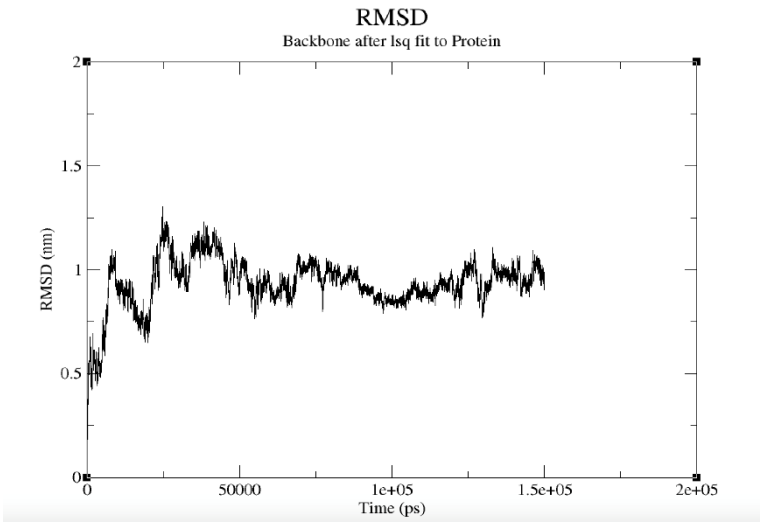

Figure 4 Time averaged distance between Aβ with DHMA.

4.3 Secondary Structure Change of Aβ40 Residues

The Define Secondary Structure of Protein (DSSP) analysis was performed to observe the change in the secondary structures of the 40 residues present in the Aβ protein through 150 nanoseconds as shown in Figure 5 and Figure 6. Without DHMA, most of the alpha-helix of the protein was lost at around 70 nanoseconds and beta-sheets started to form around 90 nanoseconds. When DHMA was present, the residues of the protein started to uncoil as seen from the decrease of the alpha-helical structure while at the same time the drug was able to prevent the formation of the beta-sheet structures.

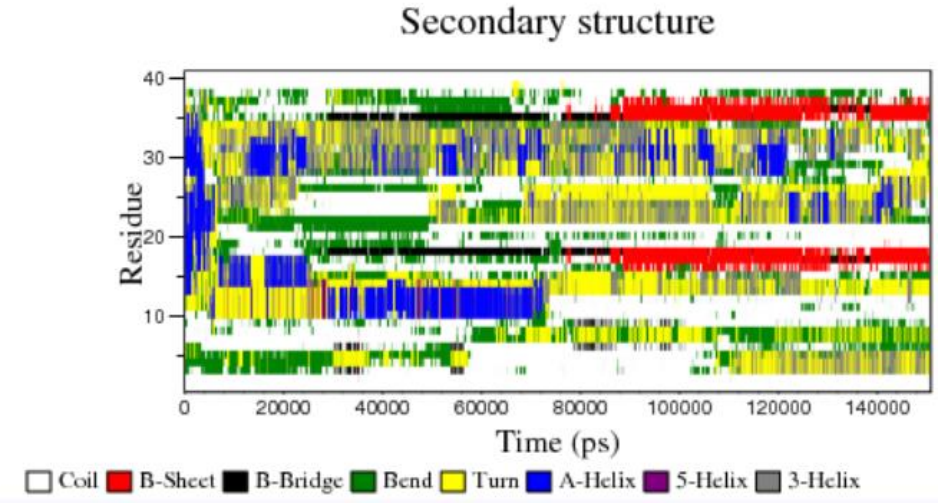

Figure 5 DSSP Analysis of Aβ40 without DHMA

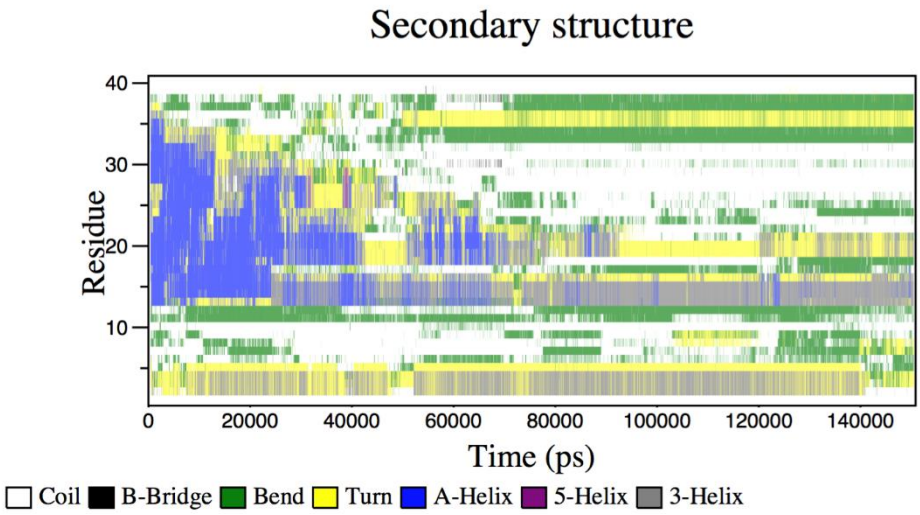

Figure 6 DSSP Analysis of Aβ40 with DHMA present

CHAPTER 5

DISCUSSION

5.1 Aβ-DHMA Complex

The change in the protein structure reveals that when the Aβ protein is by itself, it loses much of its helical structure and gradually unravels during the 150 nanoseconds duration of the simulation as observed in Figure 1. On the other hand, when DHMA is added to the Aβ protein, the protein is able to retain parts of its helical structure as shown in Figure 2. The retention of this alpha-helix is an indication that DHMA is an effective compound in preventing the formation of protein oligomers which are thought to be the main cause of Alzheimer's disease.

5.2 Conformational Change of Backbone During MD Simulation

The RMSD analysis shows that when DHMA is added to the Aβ protein its RMSD value decreases to about 0.6nm compared to just when the Aβ protein is present. This indicates that the backbone of the Aβ protein with DHMA had a fewer conformational change meaning that it was able to keep its alpha helical structure better. By retaining more of the alpha-helix, DHMA was able to prevent the transition of the Aβ protein from a helical structure to a beta-sheet structure, which prevented the formation of oligomers that are hypothesized to be the cause of Alzheimer's disease.

5.2 Conformational Change of Backbone During MD Simulation

The DSSP analysis shows that when the Aβ protein is by itself, the secondary structure of its residues start to uncoil, losing the alpha-helix which are indicated by the blue bands, and start to form beta-sheet structures which are indicated by the red bands. The formation of the beta-

sheets is what ultimately leads to the formation of the fibrils that are thought to cause plaques that result in Alzheimer's disease. On the other hand, when DHMA is present although the Aβ protein loses most of its alpha helical structures, the drug does prevent the formation of the beta-sheets, therefore indicating that it can prevent the aggregation of the fibrils.

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